

Nursery Protocol for Nurseries with Plants Infected with *Phytophthora ramorum* (Sudden Oak Death)

1. BACKGROUND AND OBJECTIVES:

In February 2002, USDA Animal and Plant Health Inspection Service (APHIS) issued a federal domestic regulation for interstate movement of *Phytophthora ramorum* (7 CFR 301.92-11). The complete text and other information may be found at USDA, APHIS' web site: <http://www.aphis.usda.gov/ppq/ispm/sod/>

In 2003, *P. ramorum* was detected in nurseries in British Columbia, Washington, Oregon and in California's Central Valley. These incidents catalyzed the need for a standard protocol for use by state and federal regulators to respond to new finds of *P. ramorum* in nurseries outside of the regulated area. To ensure that there is consistency in dealing with this disease, this Action Plan and Protocol describes the official activities performed by USDA, APHIS staff in cooperation with state agriculture regulatory officials in nurseries to respond to new infections by *P. ramorum*.

The goal of the action plan is to ensure that any infestations of this serious pathogen are consistently and effectively addressed, mitigated, and eradicated. Nursery co-operation is essential. Early detection and reporting of potential *P. ramorum* infections are critical to ensure that spread is contained. The strategies employed in this action plan are consistent with those of the European Union and of other areas where eradications are being carried out with measures that ensure rapid suppression of infection, and which prevent the spread of the pathogen.

P. ramorum infestations in nurseries may be introduced via three critical pathways.

- The movement of infected plant material from one nursery to another;
- The natural environmental movement of spores from one nursery or infected wild plants to infect plants in a nursery;
- The transmission of the disease from non-plant pathways to plant material (e.g. the introduction of infested soil, water, equipment, etc.)

2. DEFINITIONS:

Regulated Area: Regulated counties and areas as identified in regulations found at <http://www.aphis.usda.gov/ppq/ispm/sod>

Suspected infected plants: Plants with visible symptoms of *P. ramorum* infection; asymptomatic plants that are a part of an infested varietal block or derived from an infested varietal block; and/or plants that have tested positive using PCR, but have not been confirmed positive for *P. ramorum* via culturing.

Infected plants:	Plants with or without soil verified as being infected with <i>P. ramorum</i> based on isolation of the causal organism and assayed by: 1) comparative morphological determination and 2) Polymerase Chain Reaction (PCR).
Plant material at risk:	Minimally host and associated plants for <i>P. ramorum</i> as identified at http://www.aphis.usda.gov/ppq/ispm/sod (see Appendix G). Other plants are undoubtedly at risk, but their susceptibility to <i>P. ramorum</i> has not been demonstrated.
Production block (lot):	A group of plants identified as being of a unique cultivar, genus or species divided from other similar plants by a distinct, visible spatial separation that is no less than 2 meters.
Facility:	Site(s) where plants are grown, propagated, or held for sale.
Occurrence:	Presence in an area of a pest, officially reported to be indigenous or introduced, and not officially reported to have been eradicated. (FAO, 1990), FAO-CEPM, 1994.
Free from:	Of a consignment, field or place of production, without pests (or a specific pest) in numbers or quantities that can be detected by the application of phytosanitary procedures. (FAO, 1990), FAO-CEPM, 1994.
Outbreak:	An isolated pest population recently detected and expected to survive for the immediate future. FAO-CEPM, 1994.
Quarantine area:	An area within which a quarantine pest occurs and which is being officially controlled. (NAPPO, 1985) FAO-WG, 1995.
Buffer Zone:	A 10 meter radius, as measured outward from terminus of the 2 meter radius boundary that encircles the infested block.

3. TRIGGER EVENTS for REGULATORY ACTION:

- Suspect positives will trigger State action and Federal notification. A suspect is either:
 1. The receipt of plant material from a confirmed, infested nursery. See trace forwards, under Investigation below (Section 5).**OR**
 2. The detection of a plant in a nursery that the State or Federal laboratory finds positive (PCR or culture) for *P. ramorum*.
- A federally confirmed suspect will trigger federal/state action. Pending the completion of a delimiting survey, (see Regulatory below, Section 6):
 1. Shipment(s) will be placed on hold.

2. All host genera and associated host plant genera in the nursery will be placed on hold.

Identification-Definition of Official Confirmation

1. Authority and responsibilities.
 - States with quarantines for *P. ramorum* have specific responsibilities and specific authorities, as authorized by their laws and regulations, thus specific actions within and around the nursery are expected to be conducted by the State personnel with Federal support.
 - States without quarantines for *P. ramorum* will have those actions that must take place within and around the nursery be conducted under Federal authorities, in coordination with State personnel.
2. Samples for confirmation should be submitted to United States Department of Agriculture, National Plant Germplasm Laboratory (NPGPL) in Beltsville, MD (see Appendix F, Dr. Laurene Levy).
3. For those states with established quarantines for *P. ramorum*, state confirmation will be sufficient for state regulatory action. State regulatory action would be supported by APHIS, PPQ.
4. Protocols for confirmation – Two (2) techniques are required for positive confirmation of *P. ramorum*. See Appendix D.
5. Standard References. These would consist of plant pathology references available online, or in texts that provide definitive information for the diagnosis of *P. ramorum*.

4. NOTIFICATION:

- Communicate suspect finds as soon as one of the following has occurred:
 1. a positive PCR find
 2. a culture that matches the morphology for, *P. ramorum* (i.e. isolation of *P. ramorum*)
 3. A discussion with a nursery that positive stock may have been shipped to a neighboring state.
- Immediately provide notification to the owner. Notification details are provided under Regulatory Action, Section 6.
- Immediately notify the State Plant Health Director (SPHD) and the State Regulatory Official (SPRO) of the State in which the nursery is in.
- APHIS, PPQ Region Office and National Headquarters Office shall be notified. See Section F, Resources.
- Notify state plant regulatory officials (SPHD's and SPRO's) and facilities that are impacted by the trace backs and trace forwards. See Investigation, Section 5.

- **Notification of laboratory results:** laboratories need to notify the submitter, the SPHD, and the SPRO. Either the SPRO (if State authority is used) or the SPHD (if Federal authority is used) shall notify the owner of the nursery.
- **NAPPO Notification:** Notification shall be provided to NAPPO and PIM by Headquarters and CPHST, when an area is placed under restrictions and when it is released as free-from.
- **Public Notification:** The SPRO and SPHD will use state channels, including public affairs offices to make any public announcements. The SPHD will insure that the Legislative and Public Affairs is aware of the pending release, via the Region Office and National Headquarters Office.
- **Notifications provided to other States:** Infested nurseries will provide notification of any shipments of hosts/associated hosts moving out of state, to the receiving State Plant Health Director (SPHD), and/or State Plant Regulatory Official (SPRO) by fax, email, or telephone. Details of any host/associated host shipments will be provided for a one year period following the last *P. ramorum* positive plant find.

5. INVESTIGATION:

- **Trace backs.** Determine the origin of all infected plants through trace backs. After traces are completed, link to host plants, related plants, and other hosts and associated plants. Check origin of other host plants, associated plant species and neighboring plants. Trace back the plants to point of origin (propagator). Depending on the situation it may be necessary to go back further (especially foreign sources, stock that has moved around to many different nurseries). The need is to determine the site of production. As a general guideline, trace backs should extend back a minimum of one calendar year from time of initial detection.
- **Trace forwards.** Initiate trace forward investigations. Shipments made prior to the discovery of *P. ramorum* shall be identified and state SPRO's, SPHD's and receivers shall be notified of all shipments made within the prior 12 months from the first positive detection of *P. ramorum* at the nursery. Plants identified through trace forwards that have been moved into a landscape environ should be inspected and tested during optimal conditions for growth and development of *P. ramorum* symptoms.
- At the nursery, the soil, water, and growing regime should be completely documented. The loading dock, trucks, trays should be observed and the propagative, sanitation and disposal methods noted.
- **Soil.** Determine the content, origin (constituency), storage and handling of soil or growth media used in the facility. Determine if infected plant material may have contaminated soil or growth media used at the nursery.

- **Water.** Determine the source of irrigation water and where drainage water flows. Note the type of irrigation system(s) in use, areas of standing water and any safeguards against water back flow in the irrigation system, and any water treatment practices.
- **Cull piles.** Record the location of any cull piles that may be contaminated with infected plant material or associated soil. Check any cull piles for symptomatic plants, and plant material.
- **Equipment.** Determine if equipment used at the facility is shared with other facilities or field areas.
- **Fungicides.** Determine if fungicides are used on the plant materials at the nursery. If fungicides were used, then the date, material, amount and application rate should be recorded. Determine if any other type of treatments (soil amendments, fertilizers) are applied to the plants, soil or growth media.

6. REGULATORY ACTION:

Holds and Quarantine Action

- At a nursery where an infected plant is detected all host plant genera and associated host plant genera will be held until delimitation within and outside of the nursery are complete. This hold will include growth media and “any other product or article that an inspector determines to present a risk of spreading *Phytophthora ramorum*, if an inspector notifies the person in possession of the product or article that it is a restricted article”, 7CFR part 301.92 (b) (2) within the infested facility.
- For notification use PPQ form 523, Emergency Action Notification, as specified under the Plant Protection Act of 2000 and Plant Quarantine Regulations. The Required treatments and/or basic sanitary and precautionary measures (e.g. bio-containment of suspected infected material, etc.) should be included in the PPQ form 523. If the State initiated action, then the appropriate State notification would be used.
- Stop Sales notices should be placed on the nursery by the appropriate State Regulatory Official.
- Appropriate safeguarding measures must be initiated to prevent movement of the infected plant material within or from the nursery.

Survey, Delimitation, and Control within the Nursery

- **Perimeter survey.** Survey plants of all host/associated host genera located within 100-meters of the infected nursery for symptoms of disease caused by *P. ramorum*. Sample all symptomatic plants and submit to the appropriate State laboratory or other APHIS approved laboratory for diagnosis.

- **Delimiting Survey.** Inspect all hosts and associated host plant genera in the nursery. Sample those plants with typical and/or atypical symptoms. See sampling protocol (Appendix E) for sampling strategy, which is especially useful for large nurseries. All host and associated hosts beyond the 10 meter buffer, if determined through inspection and testing to be 'free from', will be released from regulatory control. In the 10 meter buffer, sample all symptomatic plants or 40 asymptomatic plants plus 2% of all plants above 100 plants. Submit samples to an officially approved laboratory for diagnostics.
- Where an infected plant(s) is found, all host and associated host plants within a contiguous block will be destroyed. The destruction block includes the infested host block and all contiguous hosts and associated hosts until you reach a 2 meter break. The two meter break occurs when there is either 2 meters with no plants or no hosts or associated host plants. Ensure destruction is carried out according to one of the methods detailed in Appendix A.
- All plants (see official regulatory list) within 10 meters of the removed plants will be held for 90 days, measured at a time when plant parts which express disease symptoms are present and environmental conditions are conducive to pathogen detection, and will be subject to two visual inspections. Samples must be taken and tested during this 90 day quarantine. Samples will be taken from symptomatic plants when available and asymptomatic (i.e., symptom-free) plants only if no symptomatic plants are present. Soil, media, and water (if applicable) should also be tested twice during the 90 day quarantine period. See Appendix D for recommended baiting system(s) for soil and water.
- Symptomatic plants may not be removed from the nursery, from any holding area in the nursery, or moved within the nursery until the nursery is officially declared free-from *P. ramorum* and removed from quarantine status.
- During the 90 day quarantine period, growers will discontinue applications of fungicides for *Phytophthora* control within the 10 meter buffer area.
- See Appendix B for basic sanitary measures that must be implemented immediately in all facilities containing a *P. ramorum* positive plant. Growers should utilize nursery best management practices (see Appendix C). Tools and other implements should only be used in the buffer area or must be disinfected prior to removal from the buffer (see Appendix B). All plant parts removed from plants within the 10 meter buffer area must be destroyed by an approved method (see Appendix A).

7. CONDITIONS FOR RELEASE:

Nursery plants that have been placed under regulatory control may be released from regulatory action by USDA, APHIS or designated authority after the 90 day quarantine if the following can be demonstrated:

- There are no additional detections of *P. ramorum* in nursery stock.
- Water and potting media have also tested negative for *P. ramorum*.

If water is infested, treatment is imposed (see Appendix A) the 90 day quarantine period re-starts. Potting media and water must be retested within the new 90 day quarantine period. If water is found to be positive, then any portion of the nursery that has been irrigated with *P. ramorum* infested water is placed on hold. The goal is to achieve an area-free – from status.

Criteria for release of nursery and for phytosanitary certification of plants on hold will include two distinct diagnostic procedures as outlined below.

- Culture isolation and PCR based confirmation
- PARP isolation + morphology confirmation
- ELISA and plating (culture isolation)

Post eradication monitoring. Nurseries that have been infested will continue to be monitored for the following two spring seasons as part of the national survey. These nurseries are not under any quarantine or regulatory action, unless additional outbreaks are detected.

APPENDIX A TREATMENTS

Incineration (burning to ash): the infected plants, associated growth media, associated containers (i.e. pots and trays), all leaf debris in and around the area where plants were stored may be disposed of by incineration at a facility or other location (e.g. on site) approved by USDA and permitted within provincial and municipal statutes. Off nursery movement must be properly safeguarded and every effort to prevent plant debris or soil from being dislodged from the plants prior to incineration should be taken. Burning may be through open burning or in an incinerator.

Deep burial: the infected plants, associated growth media, associated containers (i.e. pots and trays), all leaf debris in and around the area where plants were stored must be placed in double plastic bags of 4 mil thickness or greater and buried to a depth of no less than two meters. The material must be buried at a USDA approved site, onsite, or municipal landfill, which is expected to remain undisturbed. Every effort to prevent plant debris or soil from being dislodged from the plants should be taken.

Composting:

- As per Federal Regulations, upon publication in the Federal Register.

Treatments for Contaminated Water:

- Chlorine levels of 2mg/liter or greater has been correlated with the control of *Phytophthora* Spp. in re-circulated irrigation systems. (Hong et al. 2003)
- Chlorox (sodium hypochlorite) is labeled (EPA Reg. No 5813-50) for treatment of water (~50 ppm available chlorine) for controlling the spread of *Phytophthora lateralis* via water used for dust abatement, fire suppression and equipment cleaning.

The active ingredient level must be measured at the sprinkler head, otherwise the treatment is not effective.

APPENDIX B BIOSECURITY AND DISINFECTION METHODS

- If it is practicable, tools such as knives, pruners, water breakers, water wands and other implements used in the buffer area should only be used in the buffer area. If tools and other implements must be moved from the buffer area, then regular disinfection using an appropriate disinfectant for the control of *P. ramorum* (such as 1/9 solution of chlorine bleach or 70% or better solution of ethanol) is recommended prior to removal from the buffer area.
- A disinfectant foot bath should be placed and used by personnel entering and exiting the buffer area “hot zone” at the infested facility, where the movement of soil or plant debris on footwear is likely. The foot bath must be filled with fresh disinfectant on a daily basis. Use of disposable shoe covers may be used in lieu of a footbath, if disposed of immediately upon exit from the buffer. The disposable shoe covers must be bagged and incinerated.
- Everyone entering and leaving the facility must scrape off loose pieces of soil. Those working with, or in contact with suspected infected material (including plants), must wash hands using soap, or disinfectant immediately after completion of task.
- Host material (including leaf litter) must not be placed in compost piles or be removed from the facility as trash or in debris removal. Host material should be collected and incinerated or double bagged and deep buried in a site approved by USDA, APHIS or delegated regulatory authority.
- The tires (or other parts in contact with the soil) of vehicles must be cleaned of loose soil before leaving the infested facility.
- Do not visit other commercial operations in potentially contaminated work clothing and footwear. Where it is necessary that visitors enter the facility, the facility should ensure that every precaution is taken to prevent the movement of infected plants, contaminated soil or debris with the visitor.
- Clorox (sodium hypochlorite) is labeled (EPA Reg. No 5813-50) for treatment of water (~50 ppm available chlorine) measured at the sprinkler head) for controlling the spread of *Phytophthora lateralis* (Port Orford Cedar Root Disease) for water used for dust abatement, fire suppression and equipment cleaning.
- Treatments reported *(Erwin and Ribeiro 1996) as effective against other *Phytophthora* species include copper naphthenate for the treatment of wood surfaces, sodium hypochlorite, quaternary ammonium and hydrogen peroxide

(Zerotol) for surface disinfestation, and sodium tetrathiocarbonate, methyl bromide and chloropicrin, and metham sodium (Vapam) as soil treatments.

* Erwin, D. C. and O. K. Ribeiro. 1996. *Phytophthora Diseases Worldwide*. APS Press: St. Paul, MN.

APPENDIX C

BEST MANAGEMENT PRACTICES FOR NURSERIES

These Best Management Practices (BMP's) are designed to control or eliminate the diseases caused by *Phytophthora ramorum*.

The control of *P. ramorum* spread is based on the establishment of multiple hurdles or barriers to the pathogen with a purpose of minimizing the risk of introduction or survival of the SOD pathogen in a nursery. The BMP's assure the monitoring of the functionality of the process controls for the pathogen.

Each nursery facility is expected to review these and employ some or all of these practices depending upon their physical location and plant products that are handled. Nurseries are encouraged to incorporate these BMP's into their Standard Operating Procedures.

The BMP's have been divided into three categories:

- Exclusion
- Prevention
- Monitoring

The following BMPs should be considered for preventing the establishment or spread of diseases caused by *P. ramorum*:

Exclusion:

- No overstory or understory of known *P. ramorum* hosts on nursery growing grounds unless there is regular monitoring of those hosts.
- Confirm host stock is propagated from materials originating on site or is received from shipping nurseries (in SOD-regulated areas) under compliance agreements.
- All incoming host plants (buy-ins, transfers ...), regardless of origin, should be visually inspected for symptoms of *P. ramorum* by trained nursery personnel prior to being incorporated into the production facility.

Prevention:

- Effective fungicide program for the control of *Phytophthora* on susceptible host plants (research in progress, results pending.).

- Off load incoming shipments to an area that can be cleaned of the leafy debris. Sweep debris from the receiving pad and the delivery truck; collect debris and bag for disposal.
- Avoid product returns of nursery stock from a receiver in a regulated area. If unavoidable, contact your State Regulatory Official (if in California your County Agricultural Commissioner) prior to accepting the nursery stock return.

Monitoring:

- Nursery personnel should attend one or more SOD trainings. Training is available through the California Oak Mortality Task Force, USDA Forest Service, California Department of Food and Agriculture, California County Agricultural Commissioners, and other qualified personnel. SOD training may also be available through State Agriculture Departments, and Universities in other States.
- All host buy-ins should be isolated from other hosts plants and periodically inspected for symptoms of the disease over the course of a growing season.
- Monitor host and associated plants in surrounding area for symptoms of *P. ramorum* in Spring/Summer.
- Identify sources of disease recognition fact sheets, and/or develop and distribute disease recognition fact sheets on host plants to educate all field nursery personnel.
- Record Keeping: Maintain accurate shipping documentation identifying product, amount, date and origin or receiver for the purpose of identifying trace backs and trace forwards.

If the disease is found in your area, these BMPs should be followed:

- Install diversion burms to prevent soil and water movement, during storm-related events, from hillsides populated with *P. ramorum* host plants.
- Place containers/pots on a soil barrier, such as gravel or on raised beds.
- Irrigation water from any source other than well or municipal water supplies should be monitored to confirm that it is free from the pathogen.
- Avoid overhead irrigation of host plants where practical. When using overhead irrigation, irrigate in the morning to allow the foliage to dry before nightfall.

**APPENDIX D
DIAGNOSTICS**

Testing: Either ELISA or PCR methodology may be used to screen samples for the presence of *P. ramorum*. Positive ELISA or PCR test results from screening must be confirmed by culturing and isolation of the pathogen and by a second PCR confirmation of the suspect culture.

Cultures:

PARP (Phytophthora selective medium) + Morphology

V8 agar medium (for identification of Phytophthora Spp. to the specific level.

Carrot agar medium (media use to promote oogonial formation).

PCR Detection

Elisa (*Phytophthora* selective diagnostic tool)
Baiting Techniques useful for extraction of *P. ramorum* from water and soil, (see Erin and Ribeiro).

APPENDIX E SAMPLING PROTOCOL

1. For invoiced host varieties that are infected or are implicated as infected based on sampling and testing done at the receiving nursery:
 - Treat each variety as the group of regulated articles for sampling purposes. Therefore, follow the protocol described in 7 CFR 301.92-11 and inspect each plant if 100 or fewer. If more than 100 then inspect 100 plus 2% of those exceeding 100. Plants to be inspected from each variety will be randomly selected from throughout the nursery. If symptomatic plants are found the inspector must collect at least one sample per symptomatic plant.
 - If fewer than 40 symptomatic plants are found adjacent to symptomatic plants, then the inspector must collect samples from non-symptomatic plants so that the total number of sampled plants is at least 40.
2. For hosts in the nursery that are the same as those invoiced above, but are not the varieties invoiced:
 - Combine all the remaining varieties of a particular host and treat them as one group of regulated articles for sampling purposes following 7 CFR 301.92-11 as described above.
 - Plants to be inspected will be randomly selected from throughout the nursery and will include as many varieties of that host as possible, excluding those already sampled.
 - For all other hosts and associated hosts in the nursery:
Combine all and treat as one group of regulated articles for sampling following 7 CFR 301.92-11 as described above.

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APPENDIX G APHIS LIST OF REGULATED AND ASSOCIATED PLANTS FOR *PHYTOPHTHORA RAMORUM*

(<http://www.aphis.usda.gov/ppq/ispm/sod/usdasodlist.html>)